SYNOPSIS

Initiation of transcription is a major step in the regulation of gene expression. A dominant theme in regulation of gene expression lies in understanding the mechanism involved in selective expression of the genes in response to external or internal stimuli. Gene regulatory proteins bind DNA at specific sites either cognate to the promoters they act upon or at a distance, thereby exerting their effect by turning on (activation) or turning off (repression) the genes. Response of these factors to the environmental signals is further achieved by the DNA binding affinity of the transcription factors that can be modulated by small ligands, concentrations of which may fluctuate in response to nutrient availability and stress.

Bacteriophages achieve a high degree of efficiency in gene expression by evolving elegant strategies of transcriptional control. *mom* gene of enterobacteriophage Mu serves as an excellent model to understand this elaborate regulation of gene expression. The gene encodes a unique DNA modification function that confers an anti-restriction phenotype to the phage genome. Though dispensable for phage growth, it is fascinating in two respects (i) a novel modification; (ii) regulation follows a complex scheme without precedence in prokaryotes. *mom* is the last gene to be expressed during the phage lytic life cycle. Premature expression of the gene is deleterious to both host and phage and hence it is under a complex regulatory network. Dam methylase, a host encoded protein acts as a positive regulator of gene expression, an example where methylation has been shown to play a positive role in regulating tranascription. OxyR, another host encoded protein negatively regulates *mom* gene expression. Dam methylation prevents the binding OxyR to its site located in the *mom* regulatory region. The regulatory interplay also involves two phage encoded proteins. C, a middle gene product is essential for transcriptional switch from middle to late genes and Com, a late gene product, for enhancing translation of *mom* mRNA. Thus, C and Com serve as transcriptional and translational activators of *mom* gene expression. P_{mom} is a weak promoter with both -10 and -35 elements away from consensus and a sub-optimal 19 bp spacer element encompassing a stretch of 6T residues that act

as negative elements. 'T stretch' is known to induce a kink in the DNA. The sub-optimal spacer region makes the promoter elements out of phase and RNAP by itself cannot bind at *mom* promoter. C protein exerts its effect in activation in a multistep mechanism. The protein binds DNA as a dimer overlapping the promoter and unwinds the DNA, realigning the promoter elements, thus recruiting the RNAP. In the next step, it enhances the promoter clearance by the enzyme, thus enhancing the rate of productive transcription.

With this prevailing knowledge on C mediated *mom* gene expression, the present thesis work describes the experiments carried out to further understand the molecular mechanism of second step activation at P_{mom}. Genetic and biochemical analysis were carried out to identify the interacting surface of C protein on RNAP. Subsequently, studies have been extended to understand the C mediated transactivation at other late promoters- *lys, I, P,* which encode for the lysis and morphogenetic functions of the phage. Finally, Mg^{2+} coordinating residues in C protein were identified to decipher the ligand induced conformational changes in the activator protein required for its transactivator function.

Chapter I, a general introduction to the thesis, deals with the detailed discussion on gene expression and its regulatory mechanisms. RNA polymerase (RNAP) being the central molecule of gene expression (transcription) its organization and assembly are discussed. With the availability of the high resolution crystal structures of bacterial RNAP, an in-depth review on RNAP structure in terms of its potential regulatory targets, conformational changes associated with the formation of a functional holoenzyme, and during its transition from initiation to elongation processes have been described. Regulation of transcription with an emphasis on activation mechanism, ligand mediated allosteric transitions in regulatory proteins and the polymerase-activator interactions are discussed citing a few examples. The chapter concludes by introducing bacteriophage Mu and *mom* gene and its regulation by C. The objectives of the thesis form the concluding section of the chapter.

Activators are capable of resurrecting defective promoters in response to cellular demands. The unusual, multistep activation of *mom* promoter (P_{mom}) by C protein involves activator mediated promoter unwinding to recruit RNA Polymerase (RNAP) and subsequent enhanced promoter clearance of the enzyme. The first step of transactivation is an interaction independent step, while the later might involve a transient interaction between C and one of the subunits of RNAP. Previous studies pointed out β′ subunit to be the most probable interaction partner. **Chapter II** comprises the genetic and biochemical studies carried out to confirm this observation. Employing a genetic screen mutations in *rpoC* gene (encoding the β′ subunit of RNAP), were isolated which result in the defective RNAP. The mutant RNAPs were assayed for their C specific activity by *in vivo* transactivation assays. Such mutants have been purified and characterized to understand their effect at different steps of C mediated *mom* gene expression during transcription initiation. The mutant RNAP had normal transcription activity with typical σ^{70} promoters but exhibited reduced productive transcription and enhanced abortive initiation on C-dependent P_{mom}. Experiments carried out to probe the interaction between C and mutant RNAP revealed that the physical interaction *per se* is not disrupted between the two proteins. Post C-mediated recruitment of RNAP to the promoter, transient interactions between the two proteins appears to induce subtle conformational changes in RNAP leading to an enhanced promoter clearance.

Transactiavtor protein C is essential for the expression of other late genes *lys, I, P* apart from *mom* during the phage life cycle. Although the mechanism of multistep activation at P_{mom} has been elucidated, little is known on the transactivation from *lys, I* and *P* promoters. **Chapter III** includes studies carried out to understand the process of activation at these promoters. Owing to the differences in their C-binding site and promoter architecture it was important to investigate the differential effect of C, if any at *lys, I , P* promoters compared to that at P_{mom}. Activators in prokaryotes are shown to stimulate different steps of transcription initiation pathway ranging from the polymerase binding to the promoters to the post recruitment steps of isomerization and promoter clearance. Effect of C at different steps of transcription initiation pathway was analysed. The results indicate that C is absolutely essential for transcription from *lys, I* and *P* promoters similar to *mom*. However, at these promoters C exerts its effect at the step of

Isomerisation from closed complex to open complex formation. Thus, C acts at a single step here and the mode of activation is different from that observed at P_{mom}.

C dimer binds DNA with high affinity and sequence specificity, to an interrupted palindromic sequence overlapping the -35 element of *mom* promoter. Mg^{2+} mediated conformational transitions in C protein are essential for its DNA binding and transactivation functions. **Chapter IV** deals with the identification of the Mg^{2+} coordinating residues in C protein. Primary sequence analyses lead to the identification of a putative metal coordinating motif (EXDXD) towards the N-terminus of the protein. These residues were subjected to site directed mutagenesis to infer their role in Mg^{2+} coordination, its associated allosteric transition required for specific interaction with DNA. Mutants showed an altered Mg^{2+} induced conformation, compromised DNA binding and reduced levels of transcription activation when compared to C protein. Though Mg^{2+} is widely used in various DNA transaction reactions, this study provides the first insights on the importance of metal-ion induced allosteric transitions in regulating transcription factor function.